

Doi: <https://doi.org/10.33003/jaat.2022.0802.10>**VITAMINS C AND E AS AMELIORATIVE AGENTS ON BODY TEMPERATURE AND SERUM CHEMISTRY OF WISTAR RATS INFECTED WITH *T. brucei brucei* (Federe strain)¹.****Joachim Joseph Ajakaiye¹; Martina Sunday Benjamin^{2*}; Yahya Shuaibu²; Aderemi Theophilus Dare³; Ramatu Lawan Bizi⁴; Bashir Adamu Kugu⁵; Asmau Asabe Mohammad⁶; Bintu; Muhammad⁷; Melemi Richard Mazadu⁷**¹Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), Nigerian Institute for Trypanosomiasis Research, No. 1 Surame Road, P. M. B 2077, U/Rimi, Kaduna, Nigeria.²Onchocerciasis Research Department, Nigerian Institute for Trypanosomiasis Research, No. 1 Surame Road, P. M. B 2077, U/Rimi, Kaduna, Nigeria.³Department of Animal Health and Husbandry, College of Agriculture and Animal Science, Ahmadu Bello University, Mando Campus, Kaduna, Nigeria.⁴Human African Trypanosomiasis Research Department, Nigerian Institute for Trypanosomiasis Research, No. 1 Surame Road, P. M. B 2077, U/Rimi, Kaduna, Nigeria.⁵Animal African Trypanosomiasis Research Department, Nigerian Institute for Trypanosomiasis (and Onchocerciasis) Research, No. 1 Surame Road, U/Rimi, P. M. B. 2077, Kaduna, Nigeria.⁶Kano State Liaison Office, Kano State Epidemiology Unit, Infectious Diseases Hospital, France Road, Kano State, Nigeria. Nigerian Institute for Trypanosomiasis Research, No. 1 Surame Road, P. M. B 2077, U/Rimi, Kaduna, Nigeria.⁷Consultancy and Extension Services Division (CESD), Nigerian Institute for Trypanosomiasis Research, No. 1 Surame Road, P. M. B 2077, U/Rimi, Kaduna, Nigeria.*Corresponding author: 08065504326, 07089038221, E-mail = marty4ben@yahoo.com**ABSTRACT**

Effect of oral administration of vitamins C and E on the body temperature and serum chemistry of Wistar rats infected with *T. brucei brucei* (Federe strain) was investigated. Twenty-five Albino Wistar rats were randomly divided into five groups of five animals each. Group I was administered with 0.5 mL normal saline only, group II was inoculated with 0.1×10^6 in 0.2 mL blood/PBS of *T. brucei brucei* only. Groups III, IV, and V were administered the same dose of parasites as in group II, and in addition, they were administered with 150 mg/kg b.w. of Vitamin C; 150 mg/kg b.w. of Vitamin E, and the combination of both Vitamins in the last group, respectively. Body temperature increased consistently in all groups except group I. However, there was a significant ($P < 0.05$) difference in all treated groups compared to group II on day 28 post-infection. ALT was significantly ($P < 0.05$) lower only in the group treated with combined vitamins compared to group II. AST and Creatinine decreased significantly ($P < 0.05$), whereas the decrease recorded for Urea was highly significant ($P < 0.01$) in all the treated groups compared to group II, values recorded for ALP followed the same pattern but in a converse direction. In conclusion, the administration of vitamins C and E, particularly in its combined form ameliorated the negative effect on the body temperature and serum biochemistry profiles of the infected rats.

Key words: Albino Wistar rats, body temperature, serum chemistry, *Trypanosoma brucei brucei* (Federe strain), vitamins C and E.

INTRODUCTION

African animal trypanosomiasis (also called *Nagana*) is a major threat to livestock development and an economically wasting disease in sub-Saharan Africa (Esievo and Saror, 1991). This disease is transmitted via blood sucking tsetse flies of the genus *Glossina* spp. (Nagamune *et al.*, 2004), and caused by *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei*; or simultaneous infection with one or more of these trypanosomes.

T. brucei brucei infection causes weight loss, as a result of the excessive production of free radicals such as: superoxides, reactive oxygen and nitrogen species, and low level glutathione (GSH) in the cells and tissues of the host organism. The free radicals thus generated permits cleavage of the sialic acids on the surface cell membrane of the host erythrocytes, leading to anaemia and cellular damage of all the affected organs (Anosa and Kaneko, 1984a; Igbokwe, 1994; Taiwo, 1998). It has also been documented that

excessive generation of free radicals is the precursor of lipid peroxidation and the attendant decrease in systemic anti-oxidants, such as the plasma level of vitamin C or ascorbic acid. (Meshnick *et al.*, 1977; Ameh, 1984; Umar *et al.*, 2000; Eze and Chukwu,, 2008).

However, it has been reported that a good nutrition for infected and uninfected control animals will cause both to grow at the same rate. (Agyemang *et al.*, 1990; Holmes *et al.*, 2000). Reports have equally been documented that the destruction of homeostasis as a result of oxidative stress may be corrected if the body system is supplemented with natural antioxidants, such as vitamin C. (Tiwari, 1999; Pietta, 2000). Since oxidative stress is prevented in the aqueous compartment and lipid bi-layer of cell membranes by anti-oxidants such as vitamins A and C, which are lipo- and hydro-soluble, respectively (Halliwell and Gutteridge, 1985). The ameliorative effect of vitamin C, vitamin E, and/or its combination on organ damage (Umar *et al.*, 2000) and severity of anaemia (Umar *et al.*, 1999) in *Trypanosoma brucei brucei* infected animals have all been reported, but from literature consulted there was paucity of information relating to the effects of this strain of trypanosome on serum chemistry.

Therefore, the objective of this study is to investigate whether or not vitamins C and E possess ameliorative effect on indicators such as the body temperature and serum chemistry of albino Wistar rats infected with *T. brucei brucei* (Federe strain).

MATERIALS AND METHOD

Experimental area

The study was carried out at the Nigerian Institute for Trypanosomiasis (and Onchocerciasis) Research (NITR) Kaduna, Nigeria, which is situated within latitude 10° 30' 00" N and longitude 7° 25' 50" E with an altitude of 614 metre above sea level.

Experimental animals

The experiment was approved by the Research Ethics Committee of NITR, and the study was conducted under protocols in the guidelines established by the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D. C. 1996). Twenty five Wistar rats weighing between 200 – 240 g at the commencement of the experiment were purchased from the rat colony of Nigerian Institute for Trypanosomiasis (and Onchocerciasis) Research, Kaduna. After a two-week period of acclimatization, the rats were left to acclimatize for another two weeks, and duly dewormed with standard drugs before commencement of the experiment. They were randomly divided into five groups of five rats each, and kept in a well-ventilated standard plastic cages as follows: Group I was neither treated nor infected (positive control), group II was intraperitoneally infected with 1×10^6 in 0.2 mL blood/PBS of inoculum containing *T. brucei brucei* (Federe strain) parasites only (negative control), while groups III, IV and V were given the same dose of inoculum and in addition they were treated orally with 150 mg/kg body weight of vitamin C; 150 mg/kg body weight of vitamin E and the combination of 150 mg/kg body weight each of vitamins C and E, respectively. The vitamins were administered once daily and throughout the experimental period (28 days). Vitamins C and E were products of a commercial company (VMD, n.v./S.A, Arendonk, Belgium) and were obtained from a veterinary commercial outlet in Kaduna, Nigeria. The animals were fed with a basal diet obtained from a commercial feed outlet (Vital Feeds Plc., Kaduna, Nigeria) and water was given *ad libitum*. Feed constituents and calculated bromatological analysis of the basal diet are shown in Table (1). The basal diet contained 11.50 MJ/kg metabolizable energy (ME) and 16.50 g of crude protein (CP), 5.50 g of calcium and 1.45 g of available phosphorus, calculated to be slightly above the nutrient requirement recommended by National Research Council (NRC, 1995).

Table 1: Composition and calculated bromatological analysis of basal diet.

Nutrients/constituents	Quantity in g/kg diet
Maize	480.0
Soya cake	175.0
Wheat offal	160.0
Fishmeal	100.0
Brewer's dried grain	20.0
Vegetables oil	25.0
Limestone	5.0
Monocalcium phosphate	10.5
Dry molasses	17.0
Sodium chloride	5.0
Pre-mix Vitamins ^(a) and Minerals ^(b)	2.5
Calculated analysis /Kg	
ME, MJ /kg	11.50
CP, g	16.50
Lysine	1.65
Methionine +Cystine, g	0.92
Tryptophan, g	0.20
Threonine, g	0.61
Ca, g	5.50
P (a), g	1.45
Na, g	0.50
Cl, g	0.50

Source: (Dale and Batal, 2006). ^(a) **Vitamin supplement per (kg) diet:** Vitamin A, 6000 IU, vitamin D₃, 5000 IU, vitamin E; 23.0 mg; vitamin k₃, 4.0 mg; thymine, 11.0 mg; riboflavin, 4.0 mg; vitamin B₁₂, 0.005 mg; pyridoxine, 1.8 mg; pantothenic acid, 20.0 mg; nicotinic acid, 35 mg; folic acid, 2.5 mg; choline chloride, 615

^(b) **Mineral supplement (mg/kg diet):** Cobalt, 0.40 mg; iron, 130 mg; copper, 5 mg; zinc, 18 mg; iodine, 1.55 mg.

Inoculation of rats with parasite

The parasites *T. brucei brucei* (Federe strain) was obtained from the cryopreserved stabilates kept in Vector and Parasitology Department of NITR, Kaduna, Nigeria. The parasite was inoculated into a clean rat which served as donor rat. Infected blood from the donor rat at peak parasitaemia, that is, 4 days post infection (DPI) was collected by means of tail pricking and diluted with cold physiological saline. The number of parasite in the diluted blood was determined through the method described by Herbert and Lumsden (1976) and a volume containing approximately 1×10^6 in 0.2 mL blood/PBS of parasites was injected intraperitoneally into each rat in groups II-V.

Measurement of body temperature

The body temperature of all the animals in all groups was measured daily between the hours of 12:00 and 15:00 pm. Briefly, each animal was gently caught and a digital thermometer with a maximum gauge of 42 °C (accuracy $\pm 0.1^\circ\text{C}$ MODE: ECT-1, MAXICOM), was

inserted 3 cm into the wall of the colorectum of each rat and at the sound of a beep, the thermometer was immediately withdrawn and values obtained recorded accordingly. Live body weight of all the animals in the groups was taken twice per week and throughout the experimental period with the aid of a standard electronic weighing balance (Salter, pocket balance, England) with a maximum calibration of 5 kg and a precision of 0.1 g.

Blood sample collection and measurement of serum profiles

Tail blood was collected daily for monitoring parasitaemia as described by Herbert and Lumsden (1976) and PCV by the micro-haematocrit method. On 28 DPI, the rats were sacrificed by humane decapitation prior anesthesia with sterile cotton impregnated with chloroform, approximately 50% of blood was collected in plain vacutainers, serum was harvested and used for the estimation of alanine amino-transferase (ALT), aspartate amino-transferase (AST) and alkaline phosphatase (ALP) activities employing the method described by (Bergmeyer *et al.*

(1978) with the aid of commercial reagent kit (Gaselch aft fur Biochemica und Diagnostica, Wiesbgden, Germany).The serum samples were also used for the estimation of Urea and Creatinine by the Diacetylmonoxime and Jaffe's reactions, respectively as described by (Kaplan *et al.* (1988).

Statistical analysis

All data were presented as Means \pm SEM and analyzed by one way ANOVA. In addition differences between means were compared by means of Duncan (1955) *post-hoc* test using the SPSS version 19 statistical package and values of ($p < 0.05$) were considered significant.

RESULTS

Result of body temperature values obtained during the study period is as shown in Figure 2; There was a general significant ($p < 0.05$) increase in all infected groups compared to the positive control group. This group I showed no increase in the measured parameter throughout the experimental period as opposed to the sinusoid or undulating pattern of increase on 7 DPI, followed by a decrease on day 14 and 21 post

infection, and finally a return to the path of increase on 28 DPI in groups II, III, IV and V, respectively. However, there were significant ($p < 0.05$) decrease in all infected and vitamin treated groups either singly or in their combined form compared to the values recorded for the positive control group.

Figure 3; shows the result of serum chemistry indicators recorded during the biochemical analysis in this experiment. The levels of the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea and creatinine increased significantly ($P < 0.05$) in the infected group II compared to uninfected group I

The levels of alanine aminotransferase, aspartate aminotransferase, and urea decrease in all the treated groups compared to group II but ALP increase significantly ($P < 0.05$) in the treated groups .Likewise, there is a significant decrease in the creatine level obtained in the treated groups compared to group II

The combined vitamins(C+E) show a significant ($P < 0.05$) decrease in all the levels of serum chemistry analysed except alkaline phosphatase which shows a significant ($P < 0.05$) increase.

Table 2: Body Temperature of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with oral vitamins C and E (Means \pm SEM, n = 5).

Group	Uninfected untreated I	Infected Untreated II	Infected + Vit. C III	Infected + Vit. E IV	Infected + Vit. C and E V
Initial	37.54 \pm 0.16	37.44 \pm 0.13	37.60 \pm 0.21	37.54 \pm 0.19	37.64 \pm 0.15
7DPI	37.50 \pm 0.18 ^c	39.53 \pm 0.17 ^a	38.31 \pm 0.12 ^b	38.36 \pm 0.15 ^b	38.23 \pm 0.14 ^b
14DPI	37.58 \pm 0.17 ^b	38.41 \pm 0.12 ^a	37.93 \pm 0.13 ^b	37.95 \pm 0.12 ^b	37.85 \pm 0.12 ^b
21DPI	37.42 \pm 0.18 ^c	38.54 \pm 0.32 ^a	37.93 \pm 0.13 ^b	37.95 \pm 0.12 ^b	37.85 \pm 0.12 ^b
28DPI	37.51 \pm 0.17 ^c	39.85 \pm 0.17 ^a	38.48 \pm 0.12 ^b	38.64 \pm 0.13 ^b	38.33 \pm 0.13 ^b

DPI = Day post-infection; Mean values with different superscripts along the same row are statistically ($P < 0.05$) different [21].

Table 3: Serum chemistry of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with vitamins C and E (Means \pm SEM, n = 5).

Parameters	Uninfected and untreated I	Infected and untreated II	Infected + Vit C	Infected + Vit E	Infected + Vits C and E V
ALT	19.80 \pm 0.68 ^c	32.20 \pm 0.70 ^a	31.40 \pm 0.31 ^a	30.30 \pm 0.63 ^{ab}	28.50 \pm 0.85 ^b
AST	31.70 \pm 0.78 ^c	42.90 \pm 0.94 ^a	36.90 \pm 1.01 ^b	36.70 \pm 1.13 ^b	35.40 \pm 1.10 ^b
ALP	78.40 \pm 0.67 ^c	211.20 \pm 0.70 ^b	228.30 \pm 1.67 ^a	230.10 \pm 2.52 ^a	229.70 \pm 1.19 ^a
Urea	174.50 \pm 2.01 ^b	319.30 \pm 2.79 ^a	149.80 \pm 0.83 ^c	147.50 \pm 0.92 ^c	145.20 \pm 0.76 ^c
Creatinine	59.40 \pm 0.56 ^d	106.00 \pm 1.90 ^a	63.80 \pm 0.63 ^c	67.60 \pm 1.02 ^b	61.70 \pm 0.94 ^{cd}

ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; ALP= Alkaline phosphatase. Mean values with different superscripts along the same row are statistically different (P < 0.05).

DISCUSSION

In this study, the body temperature of the infected rats increased as soon as the infection was established. The increased temperature (hyperthermia) that was observed in the infected rats could be due to endogenous activities of the pyrogens released by the parasites. This finding agrees with Baracos *et al.* (1987), which reported that the release of pyrogens affect a change in the body temperature set-point in the hypothalamus. The treated groups maintained a regular mean value of body temperature in the second and third week which was significantly (P < 0.05) lower than the infected control, this could suggest that the vitamin supplementation gradually suppressed the activities of the pyrogens released by the parasites. In the fourth week, the body temperature of both infected control and treated groups increased significantly (P < 0.05) compared to the previous week. This erratic body temperature corroborates with the findings of Stephen (1986), that an unsteady body temperature is a clinical feature of trypanosomiasis resulting from a response to successive waves of parasitaemia.

It has been reported that analysis of haematological and serum/plasma chemistry parameters gives complementary information on the health status of the animal (Yakubu *et al.*, 2001; Oladele *et al.*, 2005; Yakubu *et al.*, 2005). In this experiment, there were increase in levels of Alanine amino-transaminase (ALT), aspartate amino-transaminase (AST), alkaline phosphatase (ALP), urea and Creatinine due to infection. This agrees with the findings of (Hudson (1944); Kalu *et al.* (1988); Adah *et al.* (1992); Ismaila *et al.* (2000); Umar *et al.* (2008), which reported increase in serum liver enzymes level in experimental trypanosomiasis. Increases in the level of these enzymes are indications of damage to liver, brain, and cardiac muscles as documented by Kaplan *et al.* (1988); Malomo (2000); Yakubu *et al.* (2006); Obaleye *et al.* (2007); Wurochekke *et al.* (2008). Similarly, several workers have reported

hepatocellular damage and generalized degenerative changes in other tissues and organs in trypanosomiasis (Anosa and Kaneko, 1984a,b.; Bruijn *et al.*, 1987; Pentreath and Kennedy, 2004).

These enzymes functions increased due to the production of toxin as reported by Gulec *et al.* (2006); Onyema *et al.* (2006); Oyewole and Malomo (2009). Alkaline phosphatase is a 'Marker' enzyme for plasma membrane and endoplasmic reticulum, as it has been shown that this enzyme is involved in the mediation of membrane transport (Goldfischer *et al.*, 1964) and transphorylation (Sastry. and Gupta, 1978). Elevated enzyme levels may also result from effect of trypanosome lyses resulting from the host's defence mechanisms (Awobode, 2006). The increase in ALP values obtained in the study is not in agreement with the findings of Ananthan and Kumaran (2013), which reported decrease in the activity of the enzyme in rat testis following the repeated administration of mancozeb, this could be attributed to either non-leakage of the enzyme into the extracellular fluid as a result of the intactness of the lipid bilayer of the membrane or non-inhibition of the enzyme activity by this parasite, an effect that may be attributed to the administered anti-oxidant vitamins. Similar depletion in alkaline phosphatase was also observed in the testis of rats treated with mancozeb Joshi and Gajraj (2005), and endosulphan Chitra *et al.* (1999) which reported that the reduction in the activity of this enzyme is due to the decreased metabolic activities.

The vitamin treatment significantly lowered the disease-induced increases in ALT and AST. This agrees with the Joshi and Gajraj (2005) findings which concluded in their own study that production of free radicals and peroxides in rats infected and treated with vitamins C and E was minimized, thereby inducing a reduction in oxidative challenge of cellular membranes of hepatocytes.

Similarly, damage to renal structures was obvious by the increases in serum urea and creatinine and these were significantly ($P < 0.05$) prevented due to the immunity conferred on them by the vitamins against the oxidative stress, caused by trypanosome-generated free radicals.

The combined vitamins was more effective compared to the singular vitamin administration in lowering the increases observed in the serum levels of ALT, AST, urea and creatinine; this could be attributed to the function of vitamin E being able to substantially interact with (n-3) fatty acid thereby boosting the immune system as reported by Slater (1984) and Fritsche et al. (1992).

CONCLUSION

In conclusion, administration of vitamins C and E singly, but more particularly in its combined form ameliorated the negative effect caused by *T. brucei* on the experimental animals. However, *T. brucei* infection resulted in increase in the serum chemistry concentrations of ALT, AST, ALP, Urea and Creatinine in albino Wistar rats. The importance of the increase in serum levels of the observed biochemical molecules in all infected groups on the pathogenesis of African trypanosomiasis could give an insight into the deleterious effects of this etiology on the liver, spleen and kidney which could result into their malfunctions. Therefore, increase in all the observed serum levels may be one of the pathophysiological mechanisms in the development of some of the reported organ damage observed in trypanosome infected animals. Furtherance to our conclusion, we suggest the inclusion of both anti-oxidant vitamins in the traditional drug therapy of African trypanosomiasis as an interim measure against increased drug resistance by the parasites.

REFERENCES

- Adah, M. J., Otesile, E. B. and Joshua, R. A. (1992). "Changes in level of transaminases in goats experimentally infected with *T. congolense*". *Rev d' Elev. Medecine Veterinaire Pays Tropicale*, 45(3-4): 284-286.
- Agyemang, K., Dwinger, R. H., Touray, B. N., Jeannin, P., Fofana, D. and Grieve, A. S. (1990). "Effects of nutrition on degree of anaemia and liveweight changes in N'Dama cattle infected with trypanosome"s. *Livestock Production Science*, 26: 39-51.
- Ameh, D. A. (1984). "Depletion of reduced glutathione and the susceptibility of erythrocytes to oxidative haemolysis in rats infected with *T. brucei gambiense*. *IRCS. Journal of Medical Science*, 12: 130 ref. 6.
- Ananthan, G. and Kumaran, B. (2013). Effect of Mancozeb on the Specific activities of Testicular Phosphatases and Protective role of Vitamin C in Albino rats. *Bulletin of Environmental. Pharmacology, Life Science*, 2(7): 56-60.
- Anosa, V. O. and Kaneko, J. J. (1984a). "Pathogenesis of *T brucei* infection in deer mice (*P. maniculatus*). Ultra structural pathology of the spleen, liver, heart and kidney". *Veterinary Pathology*, 21: 229-237.
- Anosa, V. O. and Kaneko, J. J. (1984b). "Pathogenesis of *Trypanosomoma Brucei* infection in Deer Mice (*Perosomyscus maniculatus*) Light and electron microscopic study of testicular lesion," *Veterinary Pathology*, 21: 238-246.
- Awobode, H. O. (2006). "The biochemical changes induced by natural human African trypanosome infections. *African Journal of Biotechnology*, 5(9). 738-742.
- Baracos, V. E., Whitmore, W. T. and Gale, R. (1987). The metabolic cost of fever. *Canadian Journal of Physiology and Pharmacology*, 65: 1248-1254.
- Bergmeyer, H. U., Scheibe, P. and Wahlefeld, A. W. (1978). "Optimisation methods for aspartate aminotransferase and alanine aminotransferase". *Clinical Chemistry*, 24: 58-73.
- Bruijn, J. A., Oemar, B. S., Ehrick, H. H., Foidart, J. M. and Flueures, G. J. (1987). "Antibasement membrane glomerulopathy in experimental trypanosomiasis," *Journal of Immunology*, 139: 2482-2485.
- Chitra, K. C., Latchoumy, C. and Mathur, P. P. (1999). "Chronic effect of endosulfan on the testicular functions of rat". *Asian Journal of Andrology*, 1(4): 203-206.

- Dale, N. and Batal, D. A. (2006). Feedstuffs ingredients analysis table. In: 2006 (eds.) University of Georgia, Athens, GA.
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- Esievo, K.A.N. and Saror, D. (1991). Immunochemistry and trypanosomiasis *Veterinary Bulletin*, 6: 1765-777.
- Eze, J., Anene, B. and Chukwu, C. (2008). "Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index, in *Trypanosoma brucei*-infected rats". *Comparative Clinical Pathology*, 17: 67-72.
- Fritsche, K. L., Cassity, N. A. and Huang, S. C. (1992). "Dietary (n-3) fatty acid and vitamin E interactions in rats: effects on vitamin E status, immune cell prostaglandin E production and primary antibody response". *Journal of Nutrition*, 122: 1009-1018,.
- Goldfischer, S., Essner, E. and Novikoff, A. B. (1964). "The localization of phosphatase activities at the level of ultrastructure". *Journal of Histochemistry and Cytochemistry*, 12: 72-95.
- Gulec, M., Gurel, A. and Armutcu, F. (2006). "Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats," *Molecular Cell Biochemistry*, 290: 61-67.
- Halliwell, B. and Gutteridge, J. M. C. (1985). Free radicals in Biology and Medicine. Clarendon press, Oxford, pp. 346.
- Herbert, W. J. and Lumsden, W. H. R. (1976). "*Trypanosoma brucei*: A rapid matching method for estimating the host's parasitaemia", *Experimental Parasitology*, 40: 427-431.
- Holmes, P. H., Katunguka-Rwakishaya, E., Bennison, J. J., Wassink, G. J. and Parkins, J. J. (2000). "Impact of nutrition on the pathophysiology of bovine trypanosomiasis". *Parasitology*, 120: 73-85.
- Hudson, J. R. (1944). "Acute and sub-acute trypanosomiasis in cattle caused by *T. vivax*". *Journal of Comparative Pathology*, 54: 108-119.
- Igbokwe, I.O. (1994). "Mechanisms of cellular injury in African Trypanosomiasis". *Veterinary Bulletin*, 64: 611-620.
- Ismaila, A. U., Zipporah, A. T., Funnlayo, I. I., Abubakar, G. and Lawan, B. B. (2000). "The role of vitamin C administration in alleviation of organ damage in Rats infected with *Trypanosoma brucei*". *Journal of Clinical Biochemistry and Nutrition*, 28: 1-7.
- Joshi, S. C. Gulati, N. and Gajraj, A. (2005). "Evaluation of toxic impacts of mancozeb on testis in rats. *Asian Journal of Experimental Science*, 19: 73-83.
- Kalu, A. U., Ikwuegbu, O. A. and Ogbonah, G. A. (1988). "Serum protein and electrolyte levels during trypanosome infection and following treatment in the West African Dwarf goats". *Bulletin of Animal Health Production in Africa*, 37: 41-45.
- Kaplan, L. A., Szabo, L. L. and Opherin, E. K. (1988) Enzymes in clinical chemistry: Interpretation and Techniques. 3rd(ed). Lea and Feblinger, Philadelphia. pp. 182-184,
- Malomo, S. O. (2000). "Toxicological implication of ceftriaxone administration in rats," *Nigerian Journal of Biochemistry and Molecular Biology*, 15(1): 33-38.
- Meshnick, S. R., Chance, K. P. and Cerami, A. (1977). "Hemolysis of bloodstream forms of *T. brucei*". *Biochemistry and Pharmacology*, 26: 19-23.
- Nagamune, K., Acosta, A.,-Serrano, H., Uemura-Brune, C., Kunz-Renggli, Y., Maeda, M. J., Ferguson, M. J. and Kinoshita, T. (2004). "Surface Sialic acids taken from the host allow trypanosome survival in tsetse fly vectors" *Journal of Experimental Medicine*, 199(10): 1445-1450.
- NRC. (National Research Council). (1995). Nutrient requirements of laboratory animals 4th(ed) National Academy press, Washington DC USA. pp. 11-16.

- Obaleye, J. A., Akinremi, C. A. Balogun, E. A. and Adebayo, J. O. (2007). "Toxicological studies and antimicrobial properties of some Iron (III) complexes of Ciprofloxacin," *African Journal of Biotechnology*, 6(24): 2826-2832.
- Oladele, S. B., Ayo, J. O., Ogundipe, S. O. and Esievo, K. A. N. (2005). "Seasonal and sex variations in packed cell volume, haemoglobin and total protein of the guinea fowl (*Numda meleagris*) in Zaria, Northern Guinea Savannah zone of Nigeria," *Journal of Tropical Biosciences*, 5: 67-71.
- Onyema, O. O., Farombi, E. O., Emerole, G. O., Ukoha, A. I. and Onyeze, G. O. (2006) "Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats", *Indian Journal of Biochemistry and Biophysics*, 43: 20-26.
- Oyewole, O. I. and Malomo, S. O. (2009). "Toxicological assessment of oral administration of some anti-sickling agents in rats". *African Journal of Biochemical Research*, 3(2): 024-028.
- Pentreath, V. W. and Kennedy, G. E. (2004). Pathogenesis of Human African trypanosomiasis. In: *the trypanosomiasis* Eds. Maudlin, I.; Holmes, P. H. and Miles, H. CABI Publishing International. pp. 283-301.
- Pietta, P. G. (2000). "Flavonoids as antioxidants". *Journal of Nature and Production*, 63: 1035-1042.
- Sastry, A. V. and Gupta, P.K. (1978). "Effect of mercuric chloride on the digestive system of a teleost fish *Channa punctatu*"s. *Bulletin of Environmental Contamination and Toxicology*, 20: 353-360.
- Slater, T. (1984). "Free radical mechanism in tissue injury", *Journal of Biochemistry*, 222: 1-15.
- Stephen, L. E. (1986). Trypanosomiasis- A Veterinary perspective. 1st Ed. Pergamon Press, New York.
- Taiwo, V. O. (1998). "Anemia and cachexia during trypanosomiasis, putting the facts right". *Tropical Veterinarian*, 16: 85-87.
- Tiwari, A. K. (1999). "Natural product antioxidants and their therapeutic potential in mitigating peroxidative modification of lipoproteins and atherosclerosis, recent developments," *Journal of Medicine and Aromatic Plant Science*, 21: 730-741.
- Umar, A., Rumah, B. L., Bulus, S. L., Kamla, A. A. Jobin, A., Asueliman, B. I., Mazai, M. H., Ibrahim, M. A. and Isah, S. (2008). "Effects of intraperitoneal administration of vitamins C and E or A and E combinations on the severity of *Trypanosoma brucei brucei* infection in rats," *African Journal of Biochemical Research*, 2(3): 088-091.
- Umar, I. A., Toh, Z. A., Igbalajobi, F. I., Igbokwe, I. O. and Gidado, A. (1999). "The effect of orally administered vitamins C and E on severity of anaemia in *Trypanosoma brucei* infected rats." *Tropical Veterinarian*, 18: 71-77.
- Umar, I. A., Toh, Z. A., Igbalajobi, F. I., Gidado, A. and Buratai, L. B. (2000). The role of vitamin C administration in alleviation of organ damage in rats infected with *Trypanosoma brucei*". *Journal of Clinical Biochemistry and Nutrition*, 28: 1-7.
- Wurochekke, .U., Anthony, A. E. and Obidah, W. (2008). "Biochemical effects on the liver and kidney of rats administered aqueous stem bark extract of *Xemenia Americana*," *African Journal of Biotechnology*, *African Journal of Biotechnology*, 7(16): 2777-2780.
- Yakubu, M. T., Adebayo, O. J., Egwim, E. C. and Owoyele, V. B. (2005). "Increased liver alkaline phosphatase and aminotransferase activities following administration of ethanolic extract of *Khaya senegalensis* stem bark to rats," *Biokemistri*, 17(1): 27-32.
- Yakubu, M. T., Adesokan, A. A. and Akanji, M. A. (2006)." Biochemical changes in the Liver, Kidney and Serum of rat following chronic administration of cimetidine. *African Journal of Biomedical Research*, 9: 213-218.
- Yakubu, M. T., Akanji, M. A. and Salau, I. O. (2001). "Protective effect of ascorbic acid on some selected tissues of ranitidine-treated rats," *Nigerian Journal of Biochemistry and Molecular Biology*, 16(2): 177-182.